Molecular Biology

DETECTION AND ISOLATION OF THE PEPCK GENE IN *Cryptococcus neoformans*

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Cryptococcus neoformans is a major opportunistic pathogen in immunocompromised hosts and accounts for approximately 10% of AIDS-related infections. *C. neoformans* is an encapsulated fungus in which infection is initiated by inhalation into the lungs followed by hematogenous spread to the brain and meninges. The laccase gene is required for the virulence of *C. neoformans*. Our laboratory has identified 3 upstream regulators of laccase, including an RNA helicase named Varh1p. A mutant form of *C. neoformans* was constructed where *VARH1* was deleted. This mutant was compared to a wildtype form of *C. neoformans*. The wildtype form, named H99, was isolated from a patient who contracted the *C. neoformans* fungus. We extracted the RNA from both the H99 wildtype and the Δ *varh1* mutant, a Fluorescent Differential Display was then performed on the isolated RNA. A differential display is a useful method to detect differences and similarities between genes of distinct forms of fungus. This particular differential display provided us with information on which genes were (or were not) associated with the RNA helicase within the *C. neoformans* fungus. We describe RNA helicase associated genes as being "down-regulated", our differential display identified 6 "down-regulated" genes. We proceeded to subclone the fragments using the Uni-ZAP XR Library protocol. After the initial cloning we extracted the DNA plasmid from each gene, this DNA was then sequenced and cross-referenced with a DNA database known as BLAST® (Basic Local Alignment Search Tool; www.ncbi.nlm.nih.gov:80/BLAST/). Our search led us to uncover that gene numbers 7 and 8 encoded for the PEP Carboxykinase enzyme, otherwise referred to as PEPCK. It is known that PEPCK plays a key role in the gluconeogenesis pathway. The gluconeogenesis pathway is responsible for converting pyruvate into glucose through a nine-step mechanism process. PEPCK catalyzes GTP-dependent formation of phosphoenolpyruvate from oxaloacetate. Our working theory is that inhibition of the PEPCK gene might prevent utilization of substrates such as acetate and pyruvate which are known to be in high concentration in the mammalian host, thereby "starving" the *C. neoformans* fungus. Currently, research is being conducted to determine the role of PEPCK in virulence of *C. neoformans* in mice.